Supporting Information

Identification and characterization of small molecule inhibitors of a class I histone deacetylase from Plasmodium falciparum

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Methods

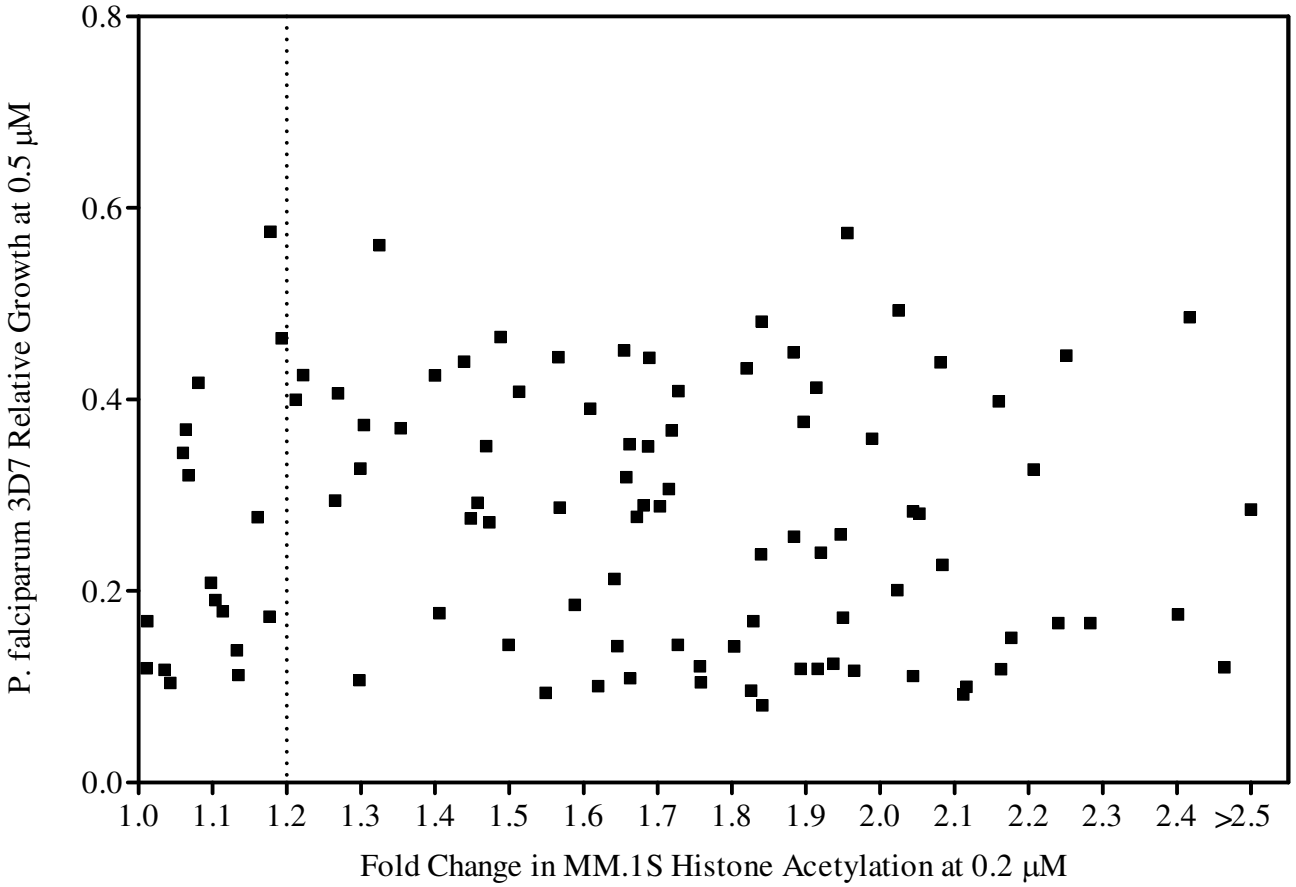
Anti-malarial activity of candidate compounds. P. falciparum 3D7 was obtained from the Malaria Research and Reference Reagent Resource Center (Manassas, VA). The parasite strains were maintained in vitro by method of Trager and Jensen.1 Reduction of P. falciparum growth in the presence of drug was assessed by the relative reduction of [3H]-hypoxanthine uptake or by staining with 4',6-diamidino-2-phenylindole (DAPI) and correlating parasite proliferation with DNA content.2-4 Candidate compounds were either assayed at a single concentration as indicated or IC50 values were calculated by non-linear regression analysis of the dose-effect curves based upon a series of 16-24 compound concentrations and Kinetic are the are the mean of three determinations ± standard deviation.

Expression and purification of pfHDAC-1. The A/T-rich sequence and unusual codon usage of the pfHDAC-1 gene warranted synthesis of a codon-optimized DNA construct (Celtek Genes). The synthetic pfHDAC-1 gene was subcloned into the pAc5.1 constitutive expression vector in-frame with a C-terminal HPC4 tag yielding pAc5.10/pfHDAC-1. Drosophila melanogaster Schneider 2 (S2) cells were grown at 27 °C in serum-containing media (Schneider's Drosophila Medium, Invitrogen). S2 cells were transfected with pAc5.10/pfHDAC-1 using the lipofectin method.5 At 72 hrs post-transfection, the cells were washed twice with PBS, re-suspended in lysis buffer (50 mM HEPES (pH 7.5), 250 mM NaCl, 0.8% Triton X-100) supplemented with Complete EDTA-free Protease Inhibitor Cocktail Tablets (Roche Applied Science), lysed by sonication, and centrifuged at 14,000×g for 20 min. The clarified lysate was incubated with anti-HPC4 resin plus 2 mM Ca2+ for 3 hrs at room temperature. The resin was then washed and bound pfHDAC-1 eluted with 0.5 mM EDTA. The protein was then buffer exchanged into PBS, concentrated, and flash frozen in liquid N2 for long-term storage at -80 oC.

pfHDAC-1 enzyme assay. Recombinant pfHDAC-1 was diluted in Buffer A (50 mM HEPES (pH 7.5), 100 mM KCl, 0.001% Tween-20, 0.05% BSA) to a final concentration of 500 nM and 30 µL was transferred to a non-binding, 384-well black plate (Corning #3654). Substrate affinity was assessed by serially diluting the candidate substrate from 1 to 200 µM in the pfHDAC-1 assay mixture. The reaction was allowed to proceed at room temperature for 15 hrs in sealed plates to minimize evaporation. The rate of product formation was determined to be linear over this time period. The fluorophore was liberated with the addition of 10 µL of Buffer A supplemented with 1 µM TSA and 500 nM trypsin. Fluorescence was monitored after 1 hr at excitation and emission wavelengths of 360 and 460 nm, respectively. Inhibitor screening was performed as above except the concentration of 1 was kept constant and in excess at 125 µM while the candidate inhibitor concentration was varied from 0.01 nM to 10 µM. The relatively high concentration of 1 may have reduced the sensitivity of the assay to competitive inhibition, however, the value chosen allowed for the selection of high potency inhibitors. Kinetic constants were calculated by non-linear regression fitting of the data to the Michaelis-Menten equation. Kinetic and inhibition values reported are the mean of three determinations ± standard deviation.

Purity Statement

All compounds were purified by either flash or reversed phase chromatography. The purity of all compounds was determined post purification by tandem high performance liquid chromatography/mass spectral (LC/MS) analyses on a Micromass Platform LCZ mass spectrometer or a Micromass Platform LCT mass spectrometer in either atmospheric pressure chemical ionization (APCI) or electrospray ionization (EI) mode after separation on a Waters Alliance separation module. The actual separations were performed on a Waters Xterra C18 column (5 µm, 2.1 x 50 mm) with a flow rate of 2 mL/min and a 2.5 min gradient of 5-100% acetonitrile in water, with a constant 0.1% formic acid buffer using a Waters 996 photodiode array detector. Purity was based on the integrated UV chromatogram (210-400 nm) and was >95% for all compounds reported with biological activity data.



Supplemental Figure 1. Comparison between the inhibition of P. falciparum proliferation relative to mock treated reference and induction of histone acetylation in mammalian MM.1S cells. Although a number of compounds were effective inhibitors of parasite growth, only seventeen were selective for P. falciparum and did not perturb histone acetylation of MM.1S cells. The dashed line indicates the criteria for selection as a hit with respect to the fold change in mammalian histone acetylation. Reference compounds SAHA (IC50 = 400nM), TSA (IC50 = 9nM) and LBH-589 (IC50 = 20nM) are not shown.

References

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